INFECTION AND IMMUNITY, Nov. 1994, p. 4831–4837
0019-9567/94/$04.00+0

Vol. 62, No. 11

Activation of Human Effector Cells by Different Bacterial Toxins (Leukocidin, Alveolysin, and Erythrogenic Toxin A): Generation of Interleukin-8

B. KÖNIG,[1] M. KÖLLER,[1] G. PREVOST,[2] Y. PIEMONT,[2] J. E. ALOUF,[3] A. SCHREINER,[1] AND W. KÖNIG[1]*

Lehrstuhl für Medizinische Mikrobiologie und Immunologie, AG Infektabwehrmechanismen, Ruhr-Universität Bochum, 44801 Bochum, Germany,[1] and Laboratoire de Toxinologie Bactérienne, Institut de Bactériologie, Faculté de Médecine, Université Louis Pasteur, 67000 Strasbourg,[2] and Unité des Toxines Microbiennes (Centre National de la Recherche Scientifique, URA 557), Institut Pasteur, 75724 Paris Cédex 15,[3] France

Received 19 April 1994/Returned for modification 9 June 1994/Accepted 7 August 1994

We analyzed the transcription and release of interleukin-8 (IL-8) from human polymorphonuclear granulocytes (PMNs) and a lymphocyte-monocyte-basophil (LMB) cell population stimulated for different time periods (30 min to 16 h) with pore-forming bacterial toxins (Panton-Valentine leukocidin [Luk-PV] and alveolysin [Alv]) as well as with the erythrogenic toxin A (ETA) as a superantigen. At high toxin concentrations (500 ng/10^7 cells), Luk-PV and Alv led to a decreased IL-8 generation from LMBs within the first 30 min; with PMNs, a slight increase in IL-8 release was observed. Under these conditions, stimulation with ETA did not lead to an altered cellular IL-8 release. At lower concentrations (5 and 0.5 ng/10^7 cells), all three toxins led to a continuous increase (over 16 h) in IL-8 release and IL-8 mRNA expression of PMNs and LMBs. Preincubation of the cells with the protein tyrosine kinase inhibitors lavendustin A and tyrphostin 25 led to a reduction of the toxin-mediated effects on IL-8 release and IL-8 mRNA expression when Luk-PV and Alv were used as stimuli. In contrast, IL-8 synthesis in cells which were stimulated with ETA was not influenced by protein tyrosine kinase inhibition. From our data, one may suggest that multiple pathways for IL-8 production are operative in human leukocytes.

Bacteria as well as bacterial products, e.g., toxins, activate inflammatory cells for a subsequent immune response. The appropriate balance of the various inflammatory mediators may be beneficial to the host; an imbalance may lead to a deleterious outcome. Recently, we have shown that two cytolytic toxins, the Panton-Valentine leukocidin (Luk-PV) and alveolysin (Alv), induce chemotactically active leukotriene B_4 (LTB_4) from human polymorphonuclear granulocytes (PMNs) (10, 11, 21). Luk-PV (37) was described as a two-component leukotoxin from *Staphylococcus aureus* V8 and was recently classified as a member of the synergohymenotropic toxins (15, 19, 25, 38). Luk-PV is only leukotoxic to human and rabbit PMNs. Alv from *Bacillus alvei* belongs to a family of cholesterol-binding cytolytic toxins which integrate into cellular membranes by complex binding to membrane sterols and thus lead to the development of hydrophilic cellular pores (8, 22, 32, 36). Cholesterol-binding toxins are predominantly produced by gram-positive human pathogenic bacteria (e.g., *Streptococcus*, *Clostridium*, and *Listeria* spp.) (3, 4, 7, 42). Like many toxins from *S. aureus* (e.g., toxic shock syndrome toxin 1 and the staphylococcal enterotoxins A and B), the erythrogenic toxin A (ETA) from *Streptococcus pyogenes* belongs to a group of superantigens. Superantigens bind to histocompatibility class II molecules and activate a large proportion of T cells through a direct interaction with the T-cell receptor (1, 24, 28). In contrast to Luk-PV and Alv, ETA did not induce LTB_4 from human PMNs but enhanced the LTB_4 generation after subsequent stimulation of the cells (20).

LTB_4 represents a mediator of the early immune response and causes the migration of eosinophils and neutrophils towards the focus of inflammation as well as the release of lysosomal enzymes (26). However, the proinflammatory cytokine interleukin-8 (IL-8) is another important chemotactic factor since it activates target leukocytes by enhancing cellular metabolism, proliferation, differentiation, and additional cytokine generation (6, 13, 16, 29, 40). Predominant cellular targets of IL-8 are neutrophils (PMNs), whose response properties include the generation of LTB_4, degranulation, and the respiratory burst.

In general, receptor activation is transmitted into the cell by distinct signal transduction pathways. Central elements of many signal transduction pathways are GTP-binding proteins and the phosphatidylinositol turnover as well as protein kinases. Recently, protein tyrosine kinases (PTKs) have been shown to be involved in the transduction of signals regulating cell growth, differentiation, and functional responses to external stimuli (23, 27). In this regard, tyrosine kinases have been shown to be involved in membrane signaling of cytokine receptors (30, 43) as well as in T-cell receptor signaling, e.g., by superantigens (2).

It was the purpose of our study (i) to elucidate the effects of Luk-PV, Alv, and ETA on human PMNs and a lymphocyte-monocyte-basophil granulocyte (LMB) cell fraction with regard to IL-8 generation and (ii) to investigate the possible involvement of PTKs in the toxin-induced activation of human leukocytes by using selective PTK inhibitors.

MATERIALS AND METHODS

Materials. Ficoll 400 was obtained from Pharmacia, Uppsala, Sweden; Macrodex (6% [wt/vol]) was from Knoll, Lud-

* Corresponding author. Mailing address: Medizinische Mikrobiologie und Immunologie, AG Infektabwehrmechanismen, Ruhr-Universität Bochum, MA01 Room 240, Universitätsstrasse 150, 44780 Bochum, Germany. Phone: (049) 0234 700 6860. Fax: (049) 0234 7094122.

wigshafen, Germany; sodium metrizoate solution (75% [wt/vol]) was from Nyegaard, Oslo, Norway; diethyl pyrocarbonate (DEPC) and *p*-nitrophenylphosphate were obtained from Sigma, Munich, Germany. The PTK inhibitors lavendustin A and 3,4,5-tri-hydroxybenzene malonodinitrile (tyrphostin 25 as described by Gazit et al. [17]) were purchased from GIBCO-BRL, Eggenstein, Germany and dissolved in a 20% aqueous solution of dimethyl sulfoxide. Lavendustin A was further diluted in phosphate-buffered saline (PBS) and applied at a concentration (50 nM) about five times higher than its 50% inhibitory concentration (11.5 nM according to the manufacturer's instructions; determined for the phosphorylation of pure epidermal growth factor receptor-associated tyrosine kinase), which proved to be most efficient in our cellular systems. Tyrphostin 25 was effective at concentrations much lower than its 50% inhibitory concentration (50 μM according to the manufacturer's instructions; determined for the autophosphorylation of the epidermal growth factor receptor) and therefore was applied at 1 μM after dilution in PBS.

Buffers and media. The buffer used throughout cellular preparation was PBS and consisted of 0.137 M NaCl, 8 mM Na_2HPO_4, 2.7 mM KH_2PO_4, and 2.7 mM KCl (pH 7.4). Before cellular incubation, RPMI 1640 (GIBCO-BRL) was used for resuspending the cells (unless stated otherwise).

Toxins. The leukotoxin used was the Luk-PV VV1 (EMBL GenBank no. 72700 [38]). The F and S components were purified to homogeneity by G. Prevost as described previously (15). The components were applied to the cell suspensions in a ratio of 1:1 (wt/wt) at the stated concentrations. Contamination of the leukocidin preparation with endotoxin could be ruled out by using the *Limulus* test. Contamination with other staphylococcal products was ruled out by G. Prevost by using enzyme-linked immunosorbent assays (ELISAs) (37a). The preparations were also devoid of detectable hemolytic activity on sheep, human, and rabbit erythrocytes, indicating the absence of contamination by hemolytic toxin. Alv and ETA were prepared and purified to homogeneity as described by Geoffroy and Alouf (18) and Alouf (5). The preparation of ETA was also devoid of hemolytic activity on sheep, human, and rabbit erythrocytes, indicating the absence of contamination by *S. pyogenes* hemolytic toxins. All toxin stock solutions were stored at −70°C and diluted in RPMI 1640 to the stated concentrations. The molecular masses were 65,000 Da for Luk-PV, 51,766 Da for Alv, and 25,805 Da for ETA.

Hemolytic activity of cytolytic toxins. Sheep erythrocytes (2% in PBS containing 2 mM calcium and 1 mM magnesium) were incubated with various concentrations (5 to 1,000 ng/ml) of Alv for 30 min at 37°C. The cell-free supernatants were measured photometrically (A_{530}). Complete lysis of the erythrocytes induced by H_2O was stated to be 100%. In contrast to Luk-PV, which is known to affect only certain leukocytes (18), Alv showed lytic activity at all concentrations tested; at 50 ng of the toxin, about 50% of the erythrocytes were lysed (1 hemolytic unit).

Preparation of the cells. Peripheral blood of healthy donors (200 ml) was mixed with 20 ml of a 0.9% NaCl solution containing 0.04 M EDTA. Centrifugation was then performed at 200 × *g* for 20 min at 20°C to separate the platelet-rich plasma from the cells. Human PMNs and LMBs were obtained after separation on a Ficoll-metrizoate gradient, followed by dextran sedimentation (9). This method leads to 95% pure and 98% viable cells. The cells were suspended to a final concentration of 2×10^7 cells per 1.2 ml in PBS or RPMI 1640.

Stimulation of the cells. PMNs or LMBs (10^7/600 μl) were incubated for 30 min to 16 h with various concentrations (500, 50, 5, or 0.5 ng) of the bacterial toxins at 37°C. Samples receiving the same volume (50 μl) of PBS (in the presence of calcium [1 mM] and magnesium [0.5 mM]) or RPMI 1640 instead of the toxins served as controls. Preincubation with PTK inhibitors (see above) was carried out for 10 min at 37°C. Cellular viability was analyzed by lactate dehydrogenase release and trypan blue exclusion assays. For low toxin concentrations (≤5 ng), no essential cytotoxic effects were observed.

Reverse transcription-PCR. Total cellular RNA was extracted essentially by the protocol of Chomczynski and Sacchi (12). For reverse transcription, 2 μg of total cellular RNA was resuspended in 20 μl of double-distilled water containing DEPC (DEPC-ddH_2O) containing 2.5 μM oligo(dT) (16-mer; GIBCO-BRL), 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM each deoxynucleoside triphosphate (GIBCO-BRL), 20 U of placental RNase inhibitor (GIBCO-BRL), and 50 U of Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL). The reaction mixture was incubated for 60 min at 37°C. The resulting cDNA samples were stored at −20°C. Ten microliters of the reaction mixture (or, in orders of magnitude, less cDNA plus appropriate amounts of DEPC-ddH_2O to ensure linearity of the PCR range) was mixed with 90 μl of DEPC-ddH_2O containing 2 mM $MgCl_2$, 50 mM Tris-HCl (pH 8.3), 1.25 U of *Thermophilus aquaticus* polymerase (U.S. Biochemicals, Cleveland, Ohio), 1 mM each deoxynucleoside triphosphate, 150 nM downstream primer, and 150 nM upstream primer. The final mixture volume of 100 μl was overlaid with 100 μl of mineral oil to prevent evaporation and then amplified in a programmable DNA thermocycler (Perkin-Elmer Cetus Corp., Überlingen, Germany) by first using a three-temperature cycle which included 5 min of denaturation at 95°C, 2 min of annealing at 50°C, and 3 min of polymerization at 72°C. Thereafter, amplification continued for 35 cycles (1 min at 95°C, 2 min at 50°C, and 3 min at 72°C) or less (25 or 15 cycles) to ensure a linear amplification range.

Primer synthesis for PCR. Primer pairs specific for IL-8 exons were designed as described by Matsushima et al. (29) and synthesized in a DNA synthesizer (PCR-MATE DNA synthesizer; Applied Bio-Systems, Foster City, Calif.). The specific sequences are: (i) 5′-primer, CTT AGA TGT CAG TGC ATA AAG ACA TAC TCC, and (ii) 3′-primer, CTC AGC CCT CTT CAA AAA CTT CTC CAC A.

Analysis of IL-8 release. Cell-free supernatants were analyzed for IL-8 by a sandwich ELISA. The specific ELISA was established with a monoclonal (mouse) anti-IL-8 (coating antibody) and an enzyme (alkaline phosphatase)-linked polyclonal (goat) anti-IL-8 (detector antibody). Both antibodies were a generous gift from M. Ceska, Sandoz Forschungsinstitut, Vienna, Austria. As the enzyme substrate, *p*-nitrophenylphosphate was used. The toxin-induced secretion rate of PMNs and LMBs was compared with the amounts of the secreted cytokines in the buffer or medium controls.

Statistical analysis. Data from different experiments with different donor cells were combined and reported as the mean ± standard deviation. The Student *t* test for independent means was used to provide a statistical analysis (P of >0.05 was considered not significant).

RESULTS

Effect of Luk-PV, Alv, and ETA on the IL-8 release from human leukocytes. We have recently shown that incubation of PMNs and LMBs with cytolytic toxins (e.g., Luk-PV and Alv; up to 500 ng/10^7 cells) leads to a significant generation of inflammatory lipid mediators (LTB_4) within 30 min in a dose-dependent manner (9, 10, 20). We analyzed IL-8 release

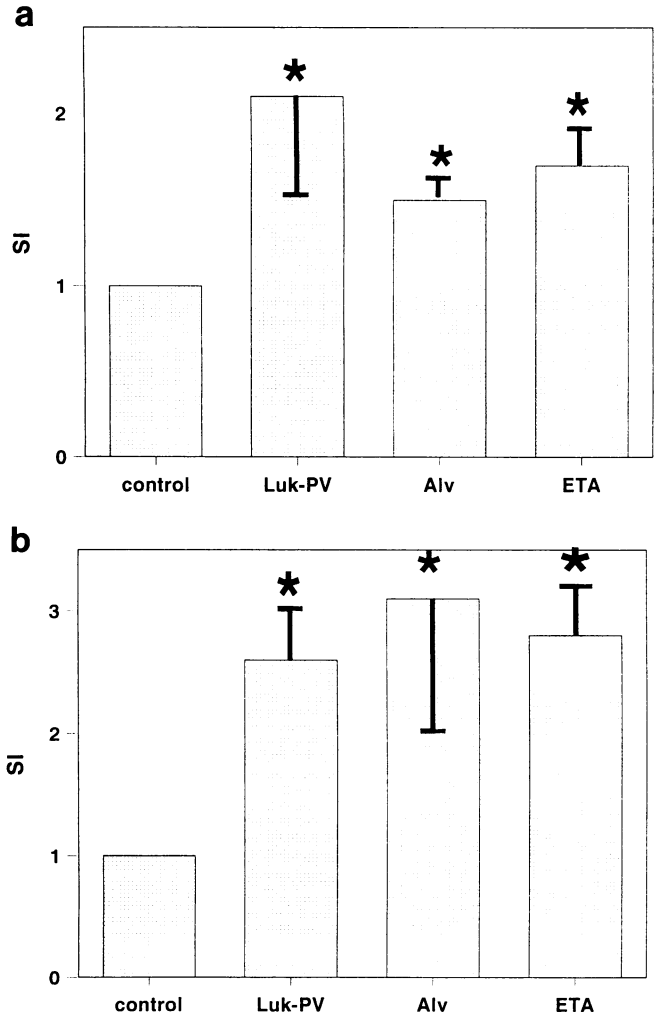

FIG. 1. IL-8 release from LMBs and PMNs induced by Luk-PV, Alv, and ETA. LMBs (a) or PMNs (b) (10^7) were stimulated with 5 ng of Luk-PV, Alv, or ETA for 16 h at 37°C. Control, cells incubated with RPMI 1640. The data represent mean values ± standard deviations (n = 3; *, significant compared with the buffer control, $P < 0.05$) and are expressed as stimulation indexes (SI). The control (incubation with RPMI 1640) is said to have an SI of 1.

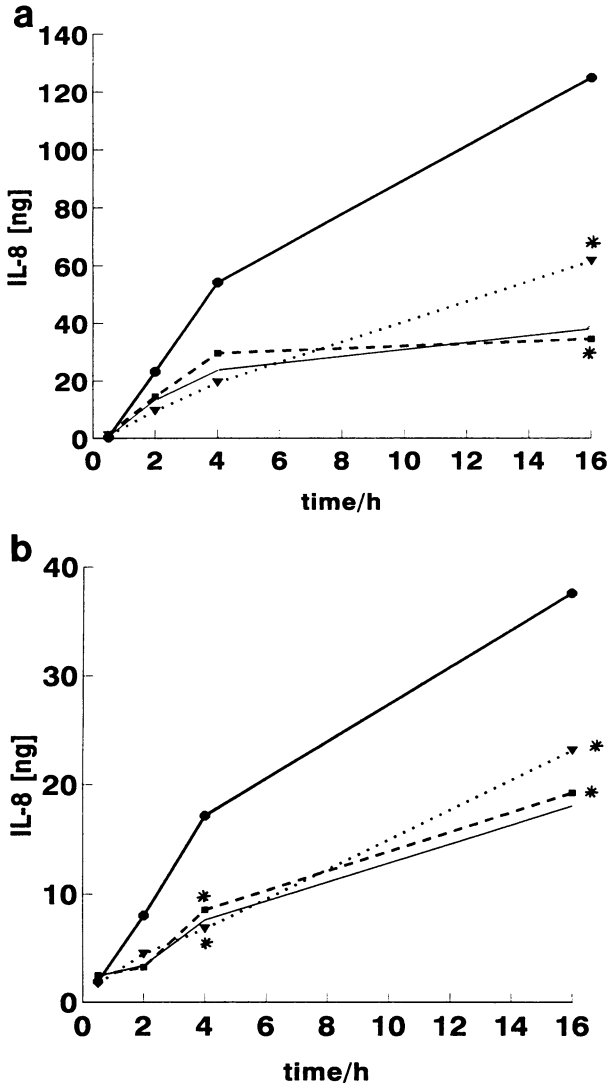

FIG. 2. Influence of lavendustin A and tyrphostin 25 on the IL-8 release from LMBs and PMNs induced by Luk-PV. LMBs (a) or PMNs (b) (10^7) were preincubated in the presence of medium (●), lavendustin A (▼), or tyrphostin 25 (■) for 10 min at 37°C; incubation then proceeded in the presence of 5 ng of Luk-PV for different time periods (30 min or 2, 4, or 16 h) at 37°C. Control, untreated cells (solid line with no markers). Values of secreted IL-8 (in nanograms) of one representative donor out of three are plotted against time (in hours). *, significantly reduced compared with cells preincubated with RPMI 1640, $P < 0.05$.

from human PMNs and LMBs stimulated with Luk-PV, Alv, and ETA (for each, at 500, 50, 5, and 0.5 ng/10^7 cells) for up to 16 h. Cellular viability was analyzed by lactate dehydrogenase release and trypan blue exclusion assays. The viability of the cells was assessed to be approximately 30, 50, 98, and 98% at concentrations of 500, 50, 5, and 0.5 ng, respectively, of Luk-PV Alv after an incubation time of 16 h; the viability of ETA-treated cells remained 98% over the concentration range tested. Thus, only data obtained with sublytic concentrations (5 ng and less) of the various toxins are presented in Fig. 1. As is apparent from Fig. 1 for a concentration of 5 ng, which was most effective for all three toxins, a marked increase in IL-8 release from LMBs (up to twofold) (Fig. 1a) and PMNs (up to threefold) (Fig. 1b) occurred after 16 h of stimulation.

Effects of PTK inhibitors (lavendustin A and tyrphostin 25) on IL-8 release. PTKs are involved in a variety of cellular processes. Therefore, we analyzed the possible involvement of PTKs in the IL-8 release induced by Luk-PV (5 and 0.5 ng/10^7 cells), Alv (5 and 0.5 ng/10^7 cells), and ETA (50, 5, and 0.5 ng/10^7 cells) for several time periods (30 min, and 2, 4, and 16 h) at 37°C. As is shown in Fig. 2 and 3 for Luk-PV and Alv at a concentration of 5 ng/10^7 cells, pretreatment with lavendustin A or tyrphostin 25 markedly decreased the toxin-induced IL-8 release from LMBs (Fig. 2a and 3a) and PMNs (Fig. 2b and 3b). The solvent used for both PTK inhibitors (dimethyl sulfoxide) alone had no effect on the toxin-induced cellular IL-8 release (data not shown). In contrast to that of Luk-PV and Alv, the ETA-induced IL-8 release was not significantly affected by either lavendustin A or tyrphostin 25 in either cell population (Fig. 4). Generally (Fig. 2 to 4), the capacity to generate IL-8 was about four times higher in LMBs than in PMNs.

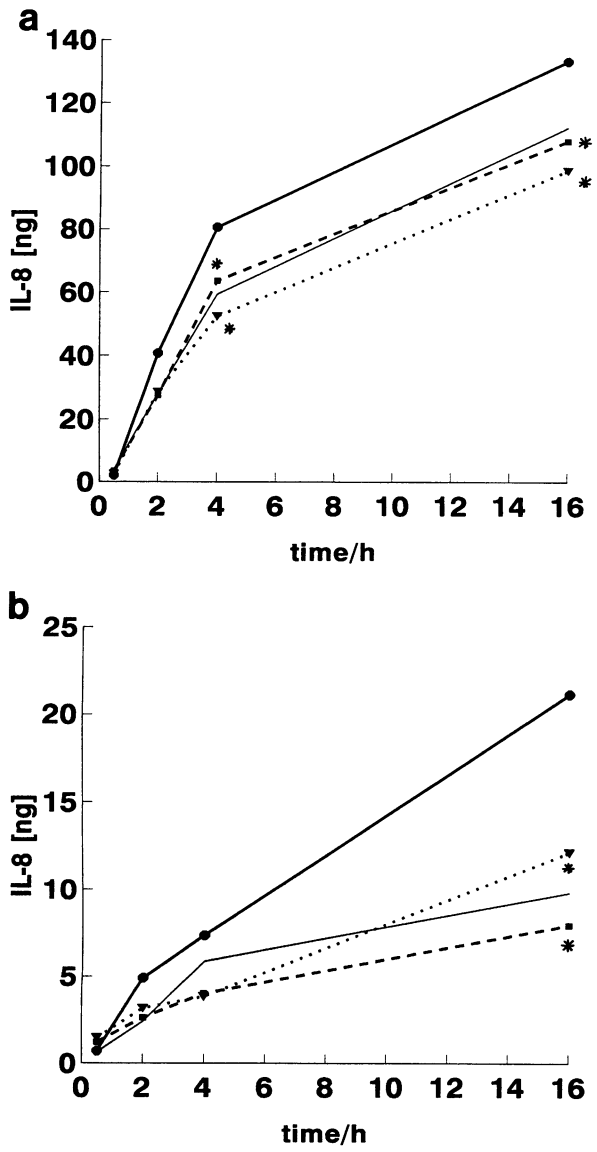

FIG. 3. Influence of lavendustin A and tyrphostin 25 on the IL-8 release from LMBs and PMNs induced by Alv. LMBs (a) or PMNs (b) (10^7) were preincubated in the presence of medium (●), lavendustin A (▼), or tyrphostin 25 (■) for 10 min at 37°C; incubation then proceeded in the presence of 5 ng of Alv for different time periods (30 min or 2, 4, or 16 h) at 37°C. Control, untreated cells (solid line with no markers). Values of secreted IL-8 (in nanograms) of one representative donor out of three are plotted against time (in hours). *, significantly reduced compared with cells preincubated with RPMI 1640, $P < 0.05$.

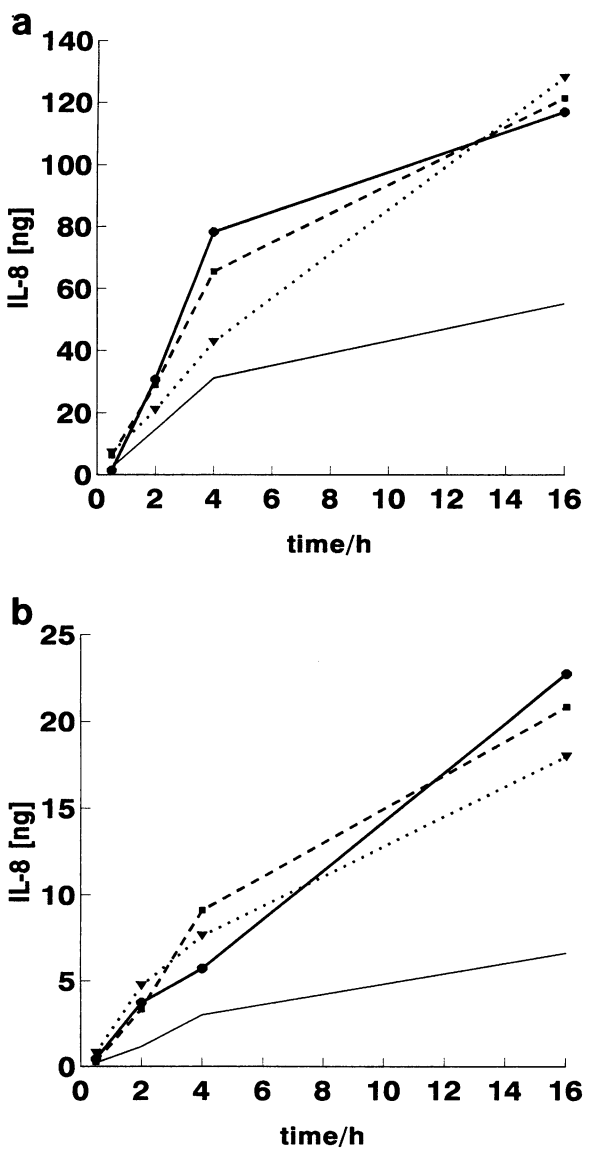

FIG. 4. Influence of lavendustin A and tyrphostin 25 on the IL-8 release from LMBs and PMNs induced by ETA. LMBs (a) or PMNs (b) (10^7) were preincubated in the presence of medium (●), lavendustin A (▼), or tyrphostin 25 (■) for 10 min at 37°C; incubation then proceeded in the presence of 5 ng of ETA for different time periods (30 min or 2, 4, or 16 h) at 37°C. Control, untreated cells (solid line with no markers). Values of secreted IL-8 (in nanograms) of one representative donor out of three are plotted against time (in hours). *, significantly reduced compared with cells preincubated with RPMI 1640, $P < 0.05$.

Effect of Luk-PV, Alv, and ETA on IL-8 transcription. Reverse transcription PCR was used to determine the effect of Luk-PV, Alv, and ETA on the level of IL-8-specific mRNA expression. The linear range of the PCR was verified by using either different amounts of cDNA in the amplification process or different cycle numbers (35, 25, or 15 cycles). Thus, identical experiments, as described above, were performed. Stimulation with Luk-PV, Alv, and ETA led to an increase in IL-8 specific mRNA expression. In contrast to those in LMBs, IL-8 mRNA levels in PMNs peaked at 4 to 8 h and then decreased over time (16 h). Figure 5 shows the IL-8-specific mRNA expression in PMNs (incubation time, 4 h) and LMBs (incubation time, 16 h) after stimulation with Luk-PV (5 ng), Alv (5 ng), and ETA (5 ng). In Fig. 6, the dose (5 and 0.5 ng)- and time (4, 8, and 16 h)-dependent effects of Luk-PV on IL-8 mRNA expression in PMNs and LMBs are presented. Similar patterns of IL-8 mRNA expression were observed for Alv and ETA (data not shown). When human PMNs or LMBs were preincubated with the PTK inhibitors lavendustin or tyrphostin 25, the Luk-PV- and the Alv-induced IL-8 mRNA expression, but not that of ETA, were downregulated in human PMNs and LMBs. In Fig. 7, the results are shown for PMNs after an incubation time of

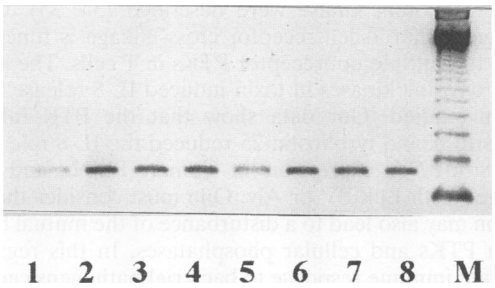

FIG. 5. IL-8-specific mRNA expression in LMBs and PMNs in the presence of Luk-PV, Alv, and ETA. Total RNA was prepared from 10^7 LMBs (lanes 1 to 4) or PMNs (lanes 5 to 8) stimulated with buffer (lanes 1 and 5), Luk-PV (5 ng; lanes 2 and 6), Alv (5 ng; lanes 3 and 7), or ETA (5 ng; lanes 4 and 8) for 16 (LMBs) or 4 (PMNs) h at 37°C. The RNA was reverse transcribed into cDNA by using oligo(dT)$_{16}$, and an aliquot was amplified with IL-8-specific primers via PCR (35 cycles). Lane M, 123-bp ladder (GIBCO-BRL).

4 h and for LMBs after an incubation time of 16 h. Lavendustin A and tyrphostin 25 did not show effects on IL-8 mRNA expression without subsequent stimulation (Fig. 7, lanes 2 and 3) compared with the buffer control (Fig. 7, lane 1).

DISCUSSION

The chemotactic potential of human effector cells consists basically of two supplementary mechanisms. Chemotactic LTB_4 is rapidly synthesized from membrane-bound arachidonic acid by the activity of a 5-lipoxygenase (11, 12, 26) and thus is part of an early immune response. IL-8 as another chemotactic factor is a proinflammatory cytokine and helps to initiate a more persistent response (6, 40, 41). Our data provide evidence that bacterial toxins (Luk-PV, Alv, and ETA) show dose-dependent effects on IL-8 release from human leukocytes. Furthermore, our data indicate that PTKs are involved to a different degree in the toxin-induced signaling pathway with regard to IL-8 release. Thus, one may suggest that multiple pathways for IL-8 may be operative in human leukocytes.

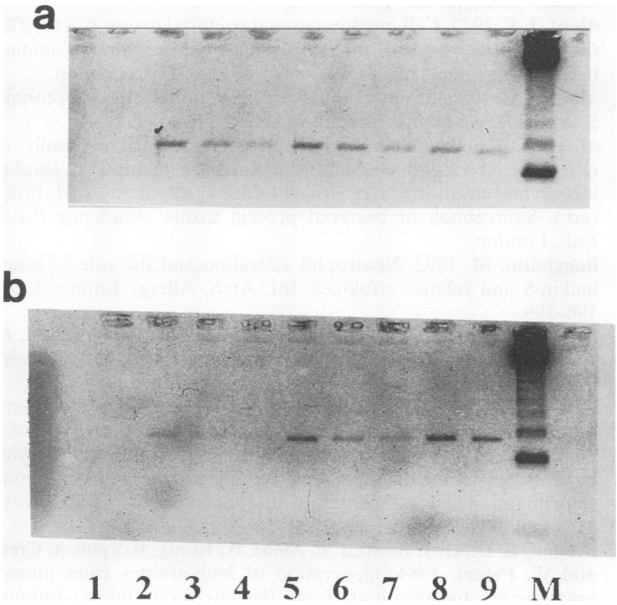

FIG. 6. IL-8-specific mRNA expression in LMBs and PMNs in the presence of Luk-PV. Total RNA was prepared from 10^7 PMNs (a) or LMBs (b) stimulated with buffer (lanes 1, 4, and 7), Luk-PV (5 ng; lanes 2, 5, and 8), or Luk-PV (0.5 ng; lanes 3, 6, and 9) for 4 (lanes 1 to 3), 8 (lanes 4 to 6), and 16 (lanes 7 to 9) h at 37°C. The RNA was reverse transcribed into cDNA by using oligo(dT)$_{16}$, and an aliquot was amplified with IL-8-specific primers via PCR (35 cycles). Lane M, 123-bp ladder (GIBCO-BRL).

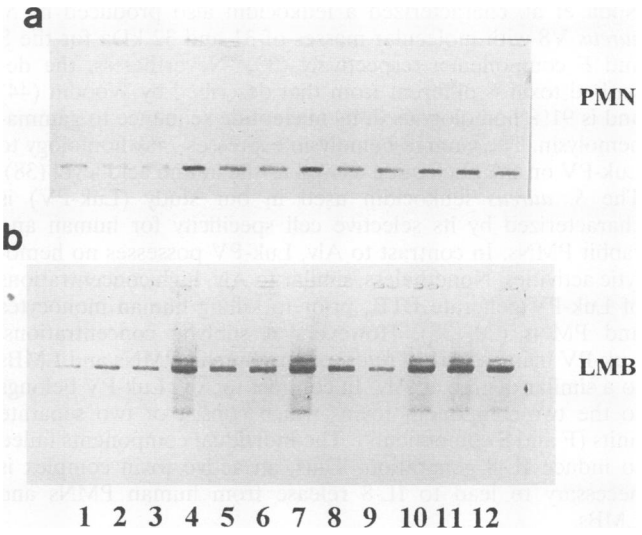

FIG. 7. Influence of lavendustin A and tyrphostin 25 on the IL-8-specific mRNA expression in LMBs and PMNs in the presence of Luk-PV, Alv, and ETA. Total RNA was prepared from 10^7 PMNs (a) or LMBs (b) (10^7) preincubated with buffer (lanes 1, 4, 7, and 10), lavendustin A (lanes 2, 5, 8, and 11), or tyrphostin 25 (lanes 3, 6, 9, and 12) (10 min, 37°C); stimulation then proceeded in the presence of buffer (lanes 1 to 3) or Luk-PV (5 ng; lanes 4 to 6), Alv (5 ng; lanes 7 to 9), or ETA (5 ng; lanes 10 to 12) for 16 (LMBs) or 4 (PMNs) h at 37°C. The RNA was reverse transcribed into cDNA by using oligo(dT)$_{16}$, and an aliquot was amplified with IL-8-specific primers via PCR (35 cycles). Lane M, 123-bp ladder (GIBCO-BRL).

The most striking biological property of Alv is its potent lytic activity towards mammalian cells. It is clearly established that cell membrane cholesterol which is located in the lipid bilayer of the cytoplasmic membrane of eukaryotic cells is the binding site of the "thiol-activated" toxin. Previously, we have shown that LTB_4 is generated by high concentrations (500 ng) of Alv within 30 min (10, 11, 21); smaller amounts of the toxin induced only negligible LTB_4 release from human PMNs. Incubation of PMNs and LMBs with a toxin concentration above 5 ng/10^7 cells finally leads to cell lysis, as was verified by lactate dehydrogenase release, trypan blue exclusion, and DNA degradation; the generation of IL-8 was not observed (data not presented). Thus, our data indicate that in contrast to LTB_4 generation, sublytic stimulation with Alv (at 5 and 0.5 ng/10^7 cells) led to a continuous induction of the IL-8 gene as evidenced by the enhanced IL-8 production from inflammatory cells for at least 16 h. Concomitant with the release of IL-8, a continuous increase in IL-8 mRNA expression was obtained in LMBs; in PMNs, maximal IL-8 mRNA expression was observed after 4 to 8 h. The addition of cholesterol, which binds Alv and thus neutralizes its effects, abolished IL-8 mRNA expression and IL-8 protein release (data not presented). Thus, the observed IL-8 generation from human PMNs and LMBs is specific to the action of Alv.

Previously, Luk-PV was described by Panton et al. (37).

Noda et al. characterized a leukocidin also produced by *S. aureus* V8 with molecular masses of 31 and 32 kDa for the S and F components, respectively (33). Nevertheless, the described toxin is different from that described by Woodin (44) and is 91% homologous in its nucleotide sequence to gamma-hemolysin. The gamma-hemolysin expresses 74% homology to Luk-PV on the nucleotide as well as the amino acid level (38). The *S. aureus* leukocidin used in our study (Luk-PV) is characterized by its selective cell specificity for human and rabbit PMNs. In contrast to Alv, Luk-PV possesses no hemolytic activities. Nonetheless, similar to Alv, high concentrations of Luk-PV generate LTB_4 prior to killing human monocytes and PMNs (20, 38). However, at sublytic concentrations, Luk-PV increased IL-8 release from human PMNs and LMBs to a similar degree as Alv. In contrast to Alv, Luk-PV belongs to the two-component toxins which consist of two separate units (F and S components). The individual components failed to induce IL-8 generation. Thus, an active toxin complex is necessary to lead to IL-8 release from human PMNs and LMBs.

The ETA has a completely different mode of action. Superantigens are known to bind to histocompatibility class II molecules and activate a large proportion of T cells through a direct interaction with the Vβ-specific T-cell receptor (1, 24). As was previously described, staphylococcal superantigens, e.g., ETA, induce the release of IL-1, tumor necrosis factor alpha, and IL-6 from macrophages via their capacity to bind major histocompatibility complex class II molecules (14, 31). Our data show that, in addition, ETA is a potent inducer of IL-8 release from human LMBs over a broad concentration range (optimal at 5 ng). In previous studies, we have shown that ETA modulates LTB_4 release from PMNs towards a subsequent stimulus; ETA by itself does not induce LTB_4 generation from human PMNs, while PMNs were stimulated for IL-8 release and IL-8-specific mRNA expression over a broad toxin concentration range (optimal at 5 ng). Similar results were obtained with other superantigens, e.g., the staphylococcal enterotoxin B as well as the toxic shock syndrome toxin 1. As was observed with ETA, both superantigens induced IL-8 mRNA expression and IL-8 protein release from human PMNs and LMBs over a broad concentration range (0.005 to 50 ng; data not shown). A mutant toxin of toxic shock syndrome toxin 1 which failed to act as a superantigen was unable to induce IL-8 generation (mRNA or protein; data not shown). Thus, we may exclude the possibility of nonspecific activation of human leukocytes by the ETA leading to IL-8 generation. Whether the activation of PMNs is mediated via major histocompatibility complex class II involvement must be further investigated. Thus, our results do not necessarily support the findings that LTB_4 formation is potentially required for the induction of IL-8 in LPS-stimulated macrophages (39).

Leukocyte responses to bacteria or bacterial products, e.g., toxins, are vital for host defense, and thus, substantial interest has been focused on defining mechanisms of signal transduction in these cells. As described above, sublytic concentrations of different toxins may induce similar pathophysiological reactions from human effector cells (e.g., the release of IL-8). Obviously, various molecular mechanisms may be involved. Among them, PTKs play an important role in receptor-mediated signaling. Early events of the action of Alv as well as Luk-PV are characteristic of a pore-forming toxin. In this regard, both toxins, Luk-PV and Alv, lead to calcium influx, which may activate, e.g., kinases. For Luk-PV, the additional involvement of phosphatidylinositol metabolism, activation of a Na^+ or K^+ ATPase, and inhibition of a cyclic AMP-dependent protein kinase were described (34, 35). Current data suggest that T-cell receptor cross-linkage is functionally coupled to multiple nonreceptor PTKs in T cells. The involvement of tyrosine kinases in toxin-induced IL-8 release has not yet been studied. Our data show that the PTK inhibitors lavendustin A and tyrphostin 25 reduced the IL-8 release and the IL-8 mRNA expression in human PMNs and LMBs challenged with Luk-PV or Alv. One must consider that PTK inhibition may also lead to a disturbance of the mutual balance between PTKs and cellular phosphatases. In this regard, an inadequate immune response to bacterial pathogens caused by an exaggerated role of activated phosphatases may also result in an impaired generation of IL-8. In contrast, both inhibitors did not modulate the ETA-induced IL-8 release and mRNA expression. Our results suggest the existence of different pathways for the generation and release of IL-8. Obviously, one cannot exclude that a PTK, which is not inhibited by lavendustin A or tyrphostin 25, is involved in the ETA-induced IL-8 release from human PMNs and LMBs.

Further studies are required to elucidate the signal transduction mechanisms (e.g., the role of distinct PTKs and phosphatases) for bacterial toxin-induced chemotactic factor generation (LTB_4 and IL-8) from human effector cells.

ACKNOWLEDGMENTS

M. Köller was supported by Deutsche Forschungsgemeinschaft. W. König was supported by Ministerium für Wissenschaft und Forschung, NRW.

REFERENCES

1. **Abe, J., J. Forrester, T. Nakahara, J. A. Lafferty, B. L. Kotzin, and D. Y. M. Leung.** 1991. Selective stimulation of human T cells with streptococcal erythrogenic toxins A and B. J. Immunol. **146:**3747–3750.
2. **Abraham, R. T., L. M. Karnitz, J. P. Secrist, and P. J. Leibson.** 1992. Signal transduction through the T-cell antigen receptor. Trends Biochem. Sci. **17:**434–438.
3. **Alouf, J. E.** 1977. Cell membranes and bacterial toxins, p. 219–270. *In* P. Cuatrecasas (ed.), The specificity and action of animal, bacterial and plant toxins. Chapman & Hall, Ltd., London.
4. **Alouf, J. E.** 1980. Streptococcal toxins (streptolysin O, streptolysin S, erythrogenic toxin). Pharmacol. Ther. **11:**661–717.
5. **Alouf, J. E., H. Knöll, and W. Köhler.** 1991. The family of mitogenic, shock-inducing and superantigenic toxins from staphylococci and streptococci, p. 367–414. *In* J. E. Alouf and J. H. Freer (ed.), Sourcebook of bacterial protein toxins. Academic Press, Ltd., London.
6. **Baggiolini, M.** 1992. Neutrophil activation and the role of interleukin-8 and related cytokines. Int. Arch. Allergy Immunol. **99:** 196–199.
7. **Bernheimer, A. W.** 1976. Sulfhydryl activated toxins, p. 85–97. *In* A. W. Bernheimer (ed.), Mechanisms in bacterial toxinology. John Wiley & Sons, Inc., New York.
8. **Bhakdi, S., and J. Tranum-Jensen.** 1988. Damage to cell membranes by pore-forming bacterial cytolysins. Prog. Allergy **40:**1–43.
9. **Böyum, A.** 1968. Isolation of mononuclear cells and granulocytes by one step centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1g. Scand. J. Clin. Lab. Invest. **21**(Suppl. 97):77–89.
10. **Bremm, K. D., H. J. Brom, J. E. Alouf, W. König, B. Spur, A. Crea, and W. Peters.** 1984. Generation of leukotrienes from human granulocytes by alveolysin from *Bacillus alvei*. Infect. Immun. **44:**188–193.
11. **Bremm, K. D., W. König, P. Pfeiffer, I. Rauschen, K. Theobald, M. Thelestam, and J. E. Alouf.** 1985. Effect of thiol-activated toxins (streptolysin O, alveolysin, and theta toxin) on the generation of leukotrienes and leukotriene-inducing and -metabolizing enzymes from human polymorphonuclear granulocytes. Infect. Immun. **50:**844–851.
12. **Chomczynski, P., and N. Sacchi.** 1987. Single step method for

RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
13. **Dibb, C. R., R. M. Strieter, M. Burdick, and S. L. Kunkel.** 1992. Expression of interleukin-8 by lipopolysaccharide-stimulated bone marrow-derived mononuclear cells. Infect. Immun. **60:**3052–3058.
14. **Fast, D. J., P. M. Schlievert, and R. D. Nelson.** 1989. Toxic shock syndrome-associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. Infect. Immun. **57:**291–294.
15. **Finck-Barbancon, V., G. Prevost, and Y. Piemont.** 1991. Improved purification of leucocidin from *Staphylococcus aureus* and toxin distribution among hospital strains. Res. Microbiol. **142:**75–85.
16. **Fujishima, S., A. R. Hoffman, T. Vu, K. J. Kim, H. Zheng, D. Daniel, Y. Kim, E. F. Wallace, J. W. Larrick, and T. A. Raffin.** 1993. Regulation of neutrophil interleukin 8 gene expression and protein secretion by LPS, TNF-alpha, and IL-1 beta. J. Cell. Physiol. **154:**478–485.
17. **Gazit, A., P. Yaish, C. Gilon, and A. Levitzki.** 1989. Tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. J. Med. Chem. **32:**2344–2352.
18. **Geoffroy, C., and J. E. Alouf.** 1983. Selective purification by thiol-disulfide interchange chromatography of alveolysin, a sulfhydryl-activated toxin of *Bacillus alvei*. Toxic properties and interaction with cholesterol and liposomes. J. Biol. Chem. **258:**9968–9972.
19. **Gladstone, G. P., and W. E. Van Heyningen.** 1957. Staphylococcal leucocidins. Br. J. Exp. Pathol. **38:**123–137.
20. **Hensler, T., M. Köller, C. Geoffroy, J. E. Alouf, and W. König.** 1993. *Staphylococcus aureus* toxic shock syndrome toxin 1 and *Streptococcus pyogenes* erythrogenic toxin A modulate inflammatory mediator release from human neutrophils. Infect. Immun. **61:**1055–1061.
21. **Hensler, T., B. König, G. Prevost, Y. Piemont, and W. König.** Unpublished data.
22. **Hugo, F., J. Reichweiss, M. Arvand, S. Krämer, and S. Bhakdi.** 1986. Use of monoclonal antibody to determine the mode of action of transmembrane pore formation by streptolysin O. Infect. Immun. **54:**641–645.
23. **Hunter, T., and J. A. Cooper.** 1985. Protein tyrosine kinases. Annu. Rev. Biochem. **54:**897–930.
24. **Kappler, J., B. L. Kotzin, L. Herron, E. W. Gelfand, R. D. Bigler, A. Boylston, S. Carrel, D. N. Posnett, Y. Choi, and P. Marrack.** 1989. Vβ-specific stimulation of human T cells by staphylococcal toxins. Science **244:**811–813.
25. **Kato, I., N. Morinaga, and R. Muneto.** 1988. Non-thiol-activated cytolytic bacterial toxins: current status. Microbiol. Sci. **5:**53–57.
26. **König, W., C. Kroegel, H. W. Kunau, and P. Borgeat.** 1981. Comparison of the eosinophil chemotactic factor (ECF) with endogenous hydroxy-eicosanoid acids of leukocytes. Monogr. Allergy **66**(Suppl. 1)**:**168–171.
27. **Levitzki, A.** 1992. Tyrphostins: tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. FASEB J. **6:**3275–3282.
28. **Marrack, P., and J. Kappler.** 1990. The staphylococcal enterotoxins and their relatives. Science **248:**705–711.
29. **Matsushima, K., K. Morishita, T. Yoshimura, S. Lavu, Y. Kobayashi, W. Lew, E. Appella, H. F. Kung, E. J. Leonard, and J. J. Oppenheim.** 1988. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J. Exp. Med. **167:**1883–1893.
30. **Miyajima, A., T. Kitamura, N. Harada, T. Yokota, and K. Arai.** 1992. Cytokine receptors and signal transduction. Annu. Rev. Immunol. **10:**295–331.
31. **Müller-Alouf, H., J. E. Alouf, D. Gerlach, C. Fitting, and J.-M. Cavaillon.** 1993. Shock-inducing cytokines produced by human immunocytes stimulated by erythrogenic toxin type A superantigen of *Streptococcus pyogenes*, abstr. 8–12. Abstr. Sixth European Workshop Conference on Bacterial Protein Toxins, Stirling, United Kingdom.
32. **Niedermeyer, W.** 1985. Interaction of streptolysin O with biomembranes: kinetic and morphological studies on erythrocyte membranes. Toxicon **23:**425–439.
33. **Noda, M., T. Hirayama, I. Kato, and F. Matsuda.** 1980. Crystallization and properties of staphylococcal leukocidin. Biochim. Biophys. Acta **633:**33–44.
34. **Noda, M., T. Hirayama, F. Matsuda, and I. Kato.** 1985. An early effect of the S component of staphylococcal leukocidin on methylation of phospholipids in various leukocytes. Infect. Immun. **50:**142–145.
35. **Noda, M., I. Kato, T. Hirayama, and F. Matsuda.** 1982. Mode of action of staphylococcal leukocidin: effects of the S and F components on the activities of membrane-associated enzymes of rabbit polymorphonuclear leukocytes. Infect. Immun. **35:**38–45.
36. **Ohno-Iwashita, Y., M. Iwamoto, K. Mitsui, S. Ando, and K. Nagai.** 1988. Protease-nicked Θ-toxin of *Clostridium perfringens* a new membrane probe with no catalytic effect reveals two classes of cholesterol as toxin-binding sites on sheep erythrocytes. Eur. J. Biochem. **176:**95–101.
37. **Panton, P. N., M. C. Camb, F. C. O. Valentine, and M. R. C. P. Lond.** 1932. Staphylococcal toxin. Lancet **i:**506–508.
37a. **Prevost, G.** Personal communication.
38. **Prevost, G., G. Supersac, D. A. Colin, P. Couppie, S. Sire, T. Hensler, P. Petiau, O. Meunier, B. Cribier, W. König, and Y. Piemont.** 1993. The new family of leucotoxins from *Staphylococcus aureus*: structural and biological properties. Zentralbl. Bakteriol. Suppl. **24:**S284–S292.
39. **Rankin, J. A., and P. Harris.** 1993. The effect of inhibition of leukotriene B_4 release on lipopolysaccharide-induced production of neutrophil attractant/activation protein-1 (interleukin-8) by human alveolar macrophages. Prostaglandins **45:**77–84.
40. **Schröder, J.-M., U. Mrowietz, and E. Christophers.** 1988. Purification and partial biologic characterization of a human lymphocyte-derived peptide with potent neutrophil-stimulating activity. J. Immunol. **140:**3534–3540.
41. **Smith, W. B., J. R. Gamble, I. Clark-Lewis, and M. A. Vadas.** 1993. Chemotactic desensitization of neutrophils demonstrates interleukin-8 (IL-8)-dependent and IL-8-independent mechanisms of transmigration through cytokine-activated endothelium. Immunology **78:**491–497.
42. **Smyth, C. J., and J. L. Duncan.** 1978. Thiol-activated (oxygen-labile) cytolysins, p. 129–183. *In* J. Jeljaszewicz and T. Wadström (ed.), Bacterial toxins and cell membranes. Academic Press Ltd., London.
43. **Vairo, G., and J. A. Hamilton.** 1991. Signalling through CSF receptors. Immunol. Today **12:**362–369.
44. **Woodin, A. M.** 1960. Purification of the two components of leukocidin from *Staphylococcus aureus*. Biochem. J. **75:**158–165.